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Original article

Effects of carbohydrate and glutamine supplementation on cytokine production by monocytes after exercise in hypoxia: A crossover, randomized, double-blind pilot study

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ABSTRACT

Objectives: The aim of this study was to evaluate the combined effects of carbohydrate (CHO) and glutamine (Gln) supplementation on cytokine production by monocytes after exercise until exhaustion performed in hypoxia.
Methods: Fifteen physically active men underwent three exercises until exhaustion with an intensity of 70% maximal oxygen intake at a simulated height of 4500 m under the following supplementation: placebo, CHO (maltodextrin 8%/200 mL for 20 min), and CHO + Gln (Gln 20 g/d for 6 d and maltodextrin 8%/200 mL for 20 min) during exercise and for 2 h of recovery. Analysis of variance for repeated measures followed by the Tukey's post hoc test was realized and $P < 0.05$ was considered statistically significant.
Results: Oxygen saturation of arterial blood (SaO_2%) decreased in the three trials compared with baseline. Two hours post-exercise, the SaO_2% was high in CHO + Gln condition compared with placebo. Two hours after exercise, interleukin (IL)-1β decreased compared with post-exercise in placebo and was lower compared with baseline in the CHO + Gln condition. Tumor necrosis factor-α decreased 2 h after exercise compared with baseline and pre-exercise in the CHO + Gln condition. No changes were observed in myeloperoxidase or IL-6 production. Two hours after exercise, Gln decreased compared with baseline and post-exercise in placebo and decreased 2 h after exercise in relation to post-exercise in the CHO condition. Gln increased post-exercise compared with pre-exercise in the CHO + Gln condition. Although erythropoietin did not change in this condition, it was high post-exercise and 2 h after exercise in the placebo condition compared with baseline and 2 h after exercise compared with baseline and pre-exercise in the CHO condition.
Conclusions: Gln supplementation for 6 d before exercise, associated with CHO supplementation during exercise, was able to revert Gln reduction after exercise and after 2 h of recovery and may have contributed to reducing tumor necrosis factor-α production, suggesting a possible anti-inflammatory effect of supplementation.

Introduction

High altitudes affect the functioning of the immune system owing to hypoxia [1]. Monocytes comprise 10% of total leukocytes in humans. They originate from hematopoietic cells in the bone marrow and enter the bloodstream continuously [2]. The cytokines produced by monocytes have autocrine, paracrine, and endocrine action, and thus they modulate inflammatory response and immune response against different stimuli, such as exposure to lipopolysaccharides (LPS) [3].

The relationship between acute or chronic exposure to hypoxia and the effects on the immune system are little understood and controversial. It is known that hypoxia reflects in a stressor environment known to cause changes in both the autonomic nervous system and endocrine function, which alters immune function [4]. A few hours of exposure to hypoxia may induce changes in various immunologic parameters, including worsening of the innate immune response, such as elevation in neutrophils and natural killer (NK) cells [1,4–6]. Increased neutrophil function has been demonstrated after 20 min of exposure to hypobaric hypoxia of 5500 m and 2 h of recovery in normoxia [7]. The production in vitro of interleukin (IL)-1β and IL-2 by mononuclear cells obtained after LPS stimulation was not affected. Additionally, elevation in the rate of phagocytosis by neutrophils has been observed, but not by monocytes during a 2-h exposure at 5500 m, with 4 h of acclimatization at 4000 m in hypoxia in a hypobaric chamber [8]. Additionally, there was a reduction in tumor

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necrosis factor (TNF)-α produced by monocytes and interferon (IFN)-ɣ secreted by CD4-like lymphocytes, in addition to increased protein expression of hypoxia-inducible factors-1α (HIF-1α) by peripheral blood mononuclear cells [8]. This information confirms that innate immune response functions under hypoxia conditions, such as phagocytosis and cytokine production, vary according to the different cell types [1].

At sea level, carbohydrate (CHO) or glutamine (Gln) supplementation alone may mitigate the effects of exhaustive exercise on immune function. Studies report the anti-inflammatory effects of CHO before and during prolonged exercise because it attenuates the blood increase of neutrophils, monocytes, cortisol, and catecholamines, improving proinflammatory IL-1β and IL-10 balance and attenuating the reduction in salivary immunoglobulin A (IgA), and T and NK cells [9]. In the case of Gln, classical studies show that this amino acid is important mainly for lymphocytes and macrophages because when the Gln level decreases, the organism suffers from transient immunosuppression [10,11], especially after strenuous exercise [12]. Immune system cells are sensitive to the Gln level for maintenance of their functions, proliferation of T and B lymphocytes, and protein synthesis including cytokines and Igs [11].

A recent study demonstrated the effect of CHO supplementation by enhancing the pro-/anti-inflammatory balance of salivary cytokines after strenuous exercise in hypoxia [13]. Additionally, supplementation can modify T-helper (Th)1/Th2 balance toward Th1 in response to hypoxia after moderate [14] and strenuous exercise [15]. On the other hand, studies on the effects of a combination of Gln and CHO supplementation on the immune system at high altitudes are still scarce.

The objective of the study was to evaluate the combined effects of CHO and Gln supplementation on cytokine production by monocytes after exercise until exhaustion performed in hypoxia. Our hypothesis was that hypoxia would impair the response of monocytes to LPS after exercise and that CHO and Gln supplementation would attenuate this worsening.

Methods

Participants

The present study included 15 healthy male participants who had performed aerobic training at least three times a week for 1 y, with the following physiologic and anthropometric characteristics: age: 26.4 ± 3.9 y; body mass: 73.7 ± 8.7 kg; height: 1.76 ± 0.02 m; body mass index (BMI): 23.7 ± 2.5 kg/m^2; maximal oxygen uptake (VO_{2max}): 50.6 ± 5.4 mL·kg·min^{-1}; maximum heart rate: 189.9 ± 8.2 bpm. The study procedures were approved by the Research Ethics Committee of the Federal University of São Paulo (UNIFESP) and are in accordance with the International Declaration of Helsinki of 1964. Individuals who had been exposed to hypoxia in the previous 6 mo, such as airplane travel or climbing above 2500 m, and those using medications that could influence outcomes, such as anti-inflammatories and antibiotics, were excluded.

Intervention

Volunteers attended the laboratory four times with 6-d intervals between each visit. On the first day, important information about the study was presented, such as the objectives and procedures to which volunteers would be submitted. At the end of the explanations, the volunteers signed the Free and Informed Consent Term. Subsequently, the volunteers were submitted to a rest and effort electrocardiogram and ergospirometry to determine the VO_{2max}. During the next weeks, the volunteers performed three exercise trials, randomized and double blind for supplementation as follows: randomization to define the order of supplementation (placebo, CHO, or CHO + Gln) was performed using a website (www.randomizer.org). Randomization and blindness were performed by a researcher from the group, but unaware of this research. The supplements and placebos had the same characteristics of color, consistency, smell, taste, and presentation. Water consumption was ad libitum throughout the experiment. The consort flow is shown in Fig. 1.The three trials were performed in hypoxia simulating an altitude of 4500 m:

- Placebo (n = 15): The volunteers consumed placebo (10 g starch + 10 g lactose) on the 6 d before testing, between 2000 and 2200. During the exercises they consumed 200 mL of a placebo solution (Clight Strawberry flavored juice, Chicago, IL, USA) each 20 min during exercise and during 2 h of recovery.
- CHO (n = 15): The volunteers consumed placebo (10 g starch + 10 g lactose) on the 6 d before testing, between 2000 and 2200. During the exercises, they consumed 200 mL of Maltodextrin 8% (Strawberry flavor - Probiotica - Laboratories, Embu das Artes, São Paulo, Brazil) each 20 min during exercise, and during 2 h of recovery.
- CHO + Gln (n = 15): The volunteers consumed 20 g of Gln (Probiotica - Laboratories) on the 6 d before testing, between 2000 and 2200. During the exercises, 200 mL of Maltodextrin 8% (Strawberry flavor - Probiotica - Laboratories) was consumed every 20 min during exercise and during 2 h of recovery.

Determination of VO_{2max}

For determination of VO_{2max} in normoxia, a treadmill test with progressive intensity was carried out, with an initial speed of 7 km/h and a load increase of 1 km·h·min^{-1} until voluntary exhaustion. Exhaustion was defined as the inability to track the speed of the treadmill for 15 s, or until the volunteers requested the interruption of the test despite encouragement to continue. A slope of 1% was used throughout the test. The respiratory parameters oxygen uptake (VO_2), carbon dioxide output (VCO_2), and respiratory quotient (QR) were measured by a gas analyzer (COSMED Model PFT - Pulmonary Function Testing - Albano Laziale - Roma, Italy) using a face mask (Hans Rudolph flow-by face mask, Shawnee, USA). All calibration procedures were performed according to the manufacturer's recommendations.

Altitude simulation

All tests were performed in a normobaric chamber that simulates altitude up to 4500 m by the change in carbon dioxide and oxygen concentration equivalent to the barometric pressure of 433 mm Hg and an inspired oxygen fraction of 13.5% O_2 (Colorado Altitude Training /12 CAT-Air Unit).

Exercise and recovery sessions

After remaining for 2 h in hypoxia, volunteers performed the exercise, with an intensity of 70% of VO_{2max} and 1% inclination, until voluntary exhaustion or a maximum of 1 h. After the end of the exercise, the volunteers remained inside the chamber in the condition of hypoxia for another 2 h. Six rest days were allowed between each trial because this time is sufficient to recover all parameters modified by hypoxia that are important for this study [16]. All exercises were performed after an overnight fast to avoid possible dietary influences and to maintain a standardized metabolic condition. Tests began at 0730 to avoid Cadian influences.

O_2 saturation in hemoglobin (SaO_2%)

SaO_2% was evaluated by a finger oximeter (FingerPulse, model M.D.300 C202, Beijing, China) at four points: before entering the chamber, after 2 h of rest in hypoxia, at the end of the exercise, and 2 h after the exercise.

Blood collection

At the same time points as SaO_2% evaluation, 40 mL of blood were withdrawn from the middle or cephalic ulnar vein. The blood was collected in EDTA tubes (Vacuette Tube) or in tubes with separator gel (Vacuette Tube). Five mL were used for cell function assays, whereas the remaining 35 mL of blood were centrifuged at 690*g* for 15 min at 4°C. The plasma or serum were extracted, aliquoted, and stored in a freezer at −80°C for further analyzes.

Biochemical and hormonal determinations

Serum Gln concentration was determined enzymatically as in a previous study [17]. Glucose was determined by the enzymatic method with kits from Labtest (Lagoa Santa, Brazil). Cortisol and erythropoietin (EPO) concentrations were determined by enzyme-linked immunosorbent assay from Arbor Assays (Ann Arbor, MI, USA) and USCN-Life Science Inc. (Wuhan, China) respectively.

Monocyte isolation

We added 3 mL Histopack 1077 and 3 mL Histopaque 1119 to the collected blood to allow sedimentation of the erythrocytes after 30 min of gradient

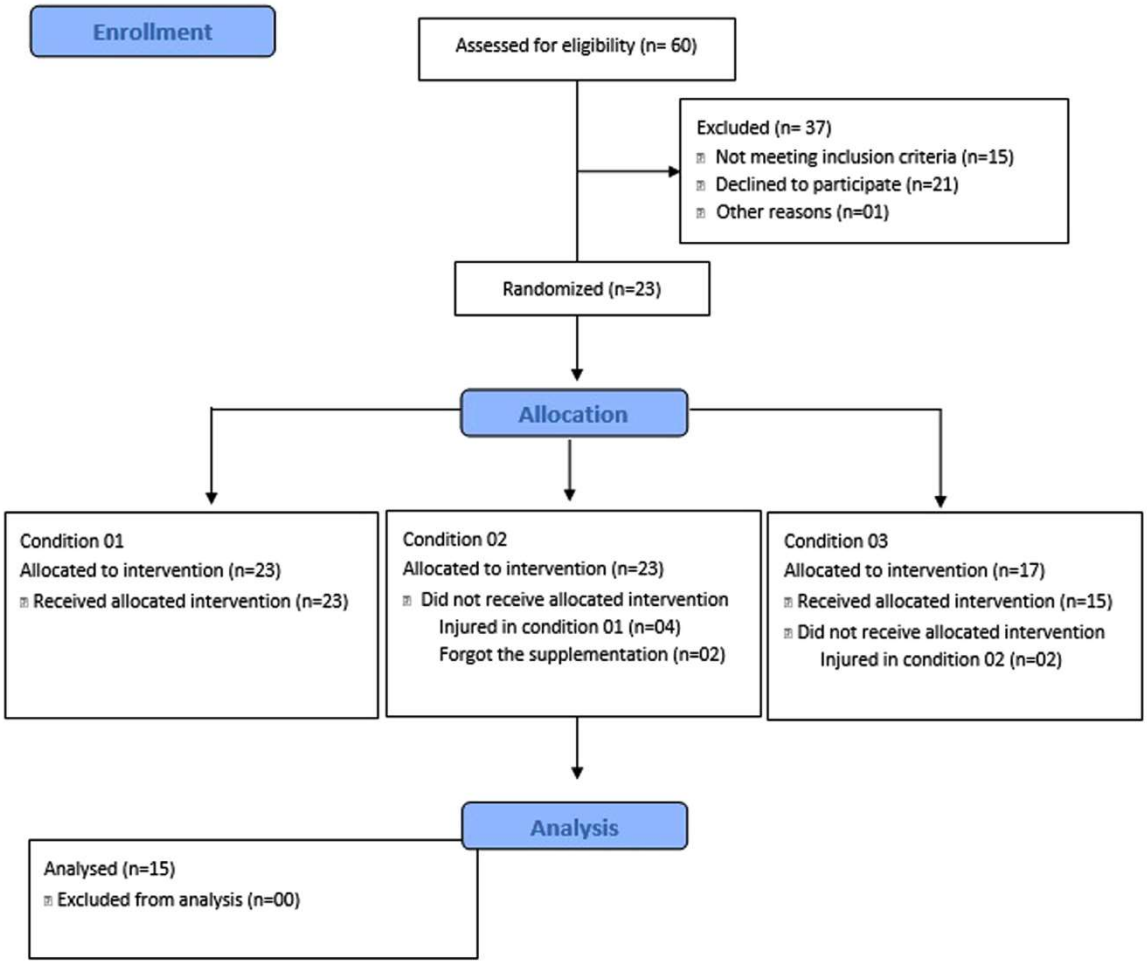

Fig. 1. Consort flow diagram, 2010.

centrifugation at a speed of 400*g* in a centrifuge without brake, at room temperature. A cloud corresponding to a portion of the leukocytes was formed above and below the neutrophils, which were transferred separately into sterile conical tubes and diluted in phosphate-buffered saline. For formation of the precipitate, the tubes were centrifuged again at a speed of 400 in a centrifuge without brake at 4°C for 10 min. Subsequently, the leukocyte pellet was resuspended in 1 mL of RPMI-1640 culture medium enriched with 10% albumin and antibiotic (streptomycin 2.5 mg/mL and penicillin 2.5 IU/mL). To separate the lymphocytes from the monocytes, incubation was performed where the monocytes are adhered to the plate, whereas the lymphocytes are dispersed in the supernatant. For this purpose, in a six-well culture dish, 2 mL of enriched RPMI-1640 culture medium and 500 μL of leukocyte cell suspension were added to the wells and incubated for 1 h in an oven at 37°C. With the monocytes adhered to the plate, the supernatant was removed, and 1.8 mL of enriched RPMI-1640 medium and 200 uL of LPS 5 ug/mL were again added to obtain a concentration of 10 ug/mL in the stimulated well, while in the well without stimulus, only 2 mL of the enriched RPMI-1640 culture medium was added. The wells were incubated for 48 h, in an oven at 37°C and 5% carbon dioxide. To collect the supernatant after 48 h, all the liquid from the plate was collected and the monocyte supernatant was centrifuged for 5 min at 400*g* at room temperature, and aliquoted in 500 μL Eppendorf tubes before being frozen at −80°C until analysis.

Cytokine production

IL-1β, IL-6, TNF-α, and myeloperoxidase (MPO) produced by stimulated monocytes were analyzed for 24 h using MilliplexMAP (Darmstadt, Germany).

Statistical analysis

The descriptive analysis was performed by mean ± SE. The normality of the data was verified using the Shapiro–Wilk test. Homogeneity and sphericity of the data were verified. To verify the group-versus-moment interactions, analysis of variance for repeated measures was used, followed by Tukey's post hoc test. The level of significance was set at $P < 0.05$. STAT version 7.0 (StataCorp, College Station, TX, USA) was used to perform the analyses.

Results

A decrease in SaO_2% at pre-exercise was observed in the placebo ($P < 0.001$), CHO ($P < 0.001$), and CHO + Gln ($P < 0.001$) groups compared with baseline. This reduction was maintained post-exercise in all three groups ($P < 0.001$; $P < 0.05$, and $P < 0.001$, respectively). Two hours after exercise, the SaO_2% was higher in the CHO + Gln group than in placebo ($P = 0.02$). Additionally, in all three groups, there was an increase 2 h after exercise in relation to post-exercise ($P < 0.001$; Fig. 2).

Regarding the production of IL-1β, no differences were observed between the treatments at the four time points. However, 2 h after exercise IL-1 decreased in the men receiving placebo relative to post-exercise ($P = 0.01$) and in those receiving CHO + Gln compared with baseline ($P = 0.03$) as shown in Figure 3. In those receiving CHO + Gln, the TNF-α production was lower 2 h after exercise compared with baseline ($P = 0.02$) and pre-exercise ($P = 0.04$; Fig. 4). Additionally, there were no statistical differences in MPO or IL-6 production, as shown in Figures 5 and 6, respectively.

Table 1 shows the results of serum Gln, glucose, cortisol, and EPO. In the placebo group, Gln was lower 2 h after exercise compared with baseline ($P = 0.01$) and post-exercise ($P = 0.02$). In those receiving CHO, the concentration of Gln was lower 2 h after

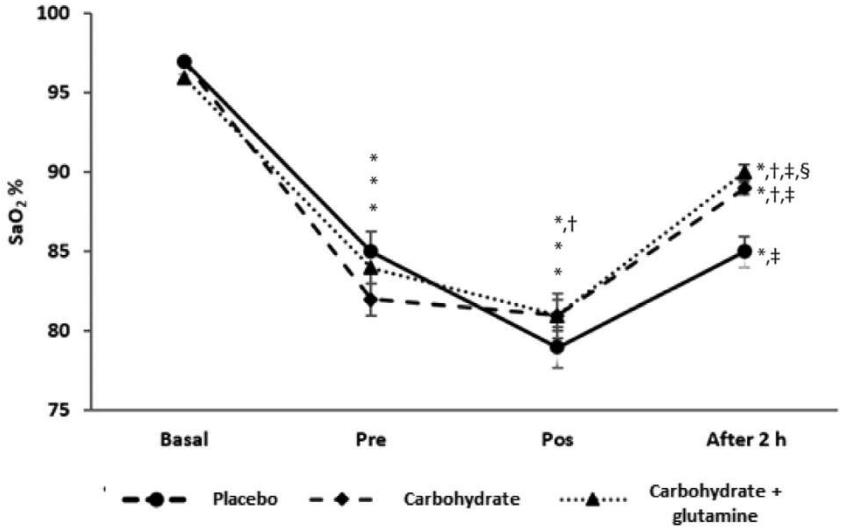

Fig. 2. Oxygen saturation (SaO2%) under placebo, carbohydrate, and carbohydrate + glutamine at baseline, pre-exercise, post-exercise, and 2 h after exercise. Results expressed as mean ± SEM. Tukey's repeated measures analysis of variance, $P < 0.05$ and N = 15. *Different in relation to baseline. †Different in relation to pre-exercise. ‡Different in relation to post-exercise. §Different in relation to hypoxia condition.

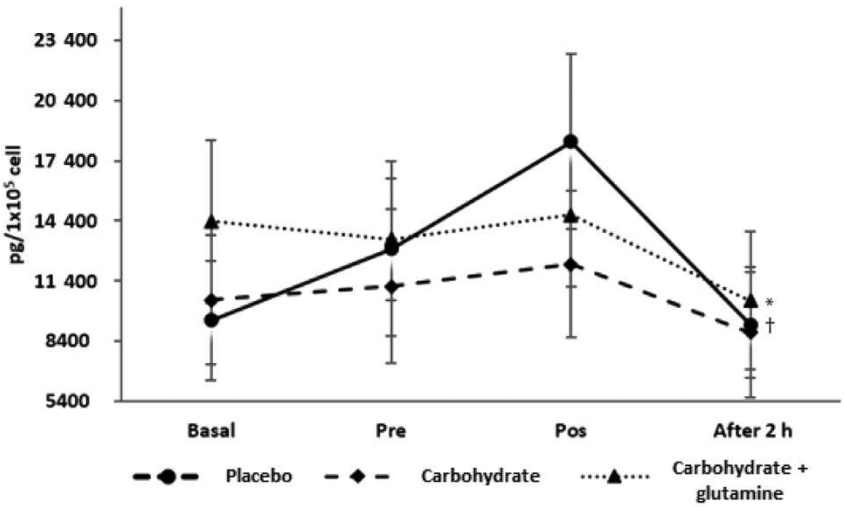

Fig. 3. Production of IL-1β under placebo, carbohydrate, and carbohydrate + glutamine at baseline, pre-exercise, post-exercise, and 2 h post-exercise. Results expressed as mean ± SEM. Tukey's repeated measures analysis of variance, $P < 0.05$ and N = 15. *Different from baseline. †Different from post-exercise.

exercise in relation to post0exercise ($P = 0.02$). In those receiving CHO + Gln, the concentration of Gln increased post-exercise in relation to pre-exercise ($P = 0.02$). In the placebo group, the glucose concentration was higher post-exercise compared with baseline ($P < 0.001$) and pre-exercise ($P = 0.01$) but was reduced 2 h after exercise in relation to post-exercise ($P = 0.01$). In those receiving CHO, the glucose concentration was higher post-exercise compared with baseline ($P < 0.001$) and pre-exercise ($P = 0.01$). The increase in relation to pre-exercise was maintained 2 h after exercise ($P < 0.01$). In those receiving CHO + Gln, the glucose concentration increased post-exercise compared with baseline ($P < 0.001$) and pre-exercise ($P = 0.01$). Two hours after exercise, glucose concentration was lower than after exercise ($P = 0.05$), but higher than pre-exercise ($P = 0.05$). In those receiving placebo, the cortisol concentration increased post-exercise in relation to baseline ($P = 0.04$) and pre-exercise ($P = 0.01$). Two hours after exercise, the cortisol was reduced compared with baseline ($P = 0.01$) and post-exercise ($P = 0.04$). Post-exercise, the cortisol concentration was higher in relation to pre-exercise ($P = 0.01$) but was reduced 2 h after exercise ($P = 0.04$) compared with post-exercise in those receiving CHO. In men receiving CHO + Gln, the concentration of cortisol was higher post-exercise ($P = 0.01$) compared with pre-exercise but was reduced 2 h after exercise in relation to post-exercise ($P = 0.01$). There was no difference in EPO concentration in those receiving CHO + Gln. In those receiving placebo, there was an increase post-exercise compared with baseline ($P = 0.02$) and this increase was maintained 2 h after exercise ($P = 0.01$). In men receiving CHO, the EPO concentration was greater 2 h after exercise than at baseline ($P = 0.01$) and pre-exercise ($P = 0.01$).

Discussion

To our knowledge, this was the first study to evaluate the effects of combined CHO and Gln supplementation on cytokine production by monocytes after strenuous exercise in hypoxia in a simulated high altitude. The main findings are that CHO + Gln increased SaO2% 2 h after exercise and may have contributed to a

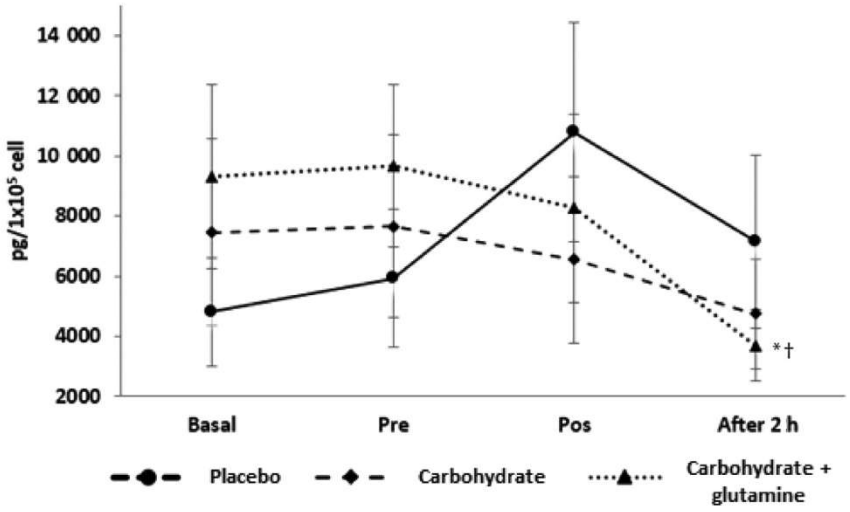

Fig. 4. Production of TNF-α under placebo, carbohydrate, and carbohydrate + glutamine at baseline, pre-exercise, post-exercise, and 2h post-exercise. Results expressed as mean ± SEM. Tukey's repeated measures analysis of variance, $P < 0.05$ and N = 15. *Different from baseline. †Different from pre-exercise.

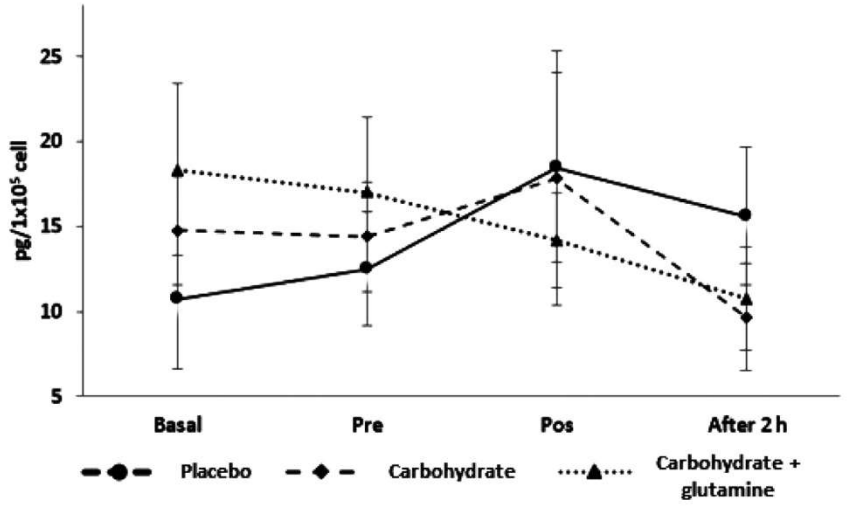

Fig. 5. MPO production under placebo, carbohydrate, and carbohydrate + glutamine at baseline, pre-exercise, post-exercise, and 2h post-exercise. Results expressed as mean ± SEM. Tukey's repeated measures analysis of variance, $P < 0.05$ and N = 15.

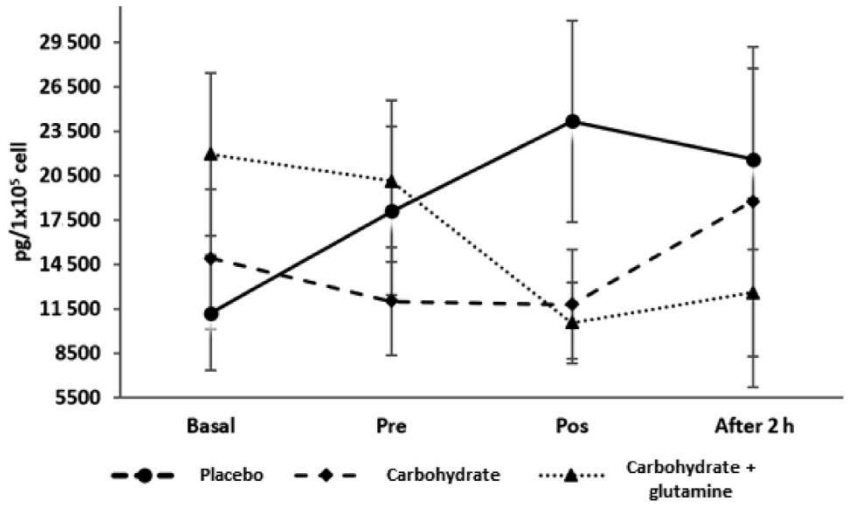

Fig. 6. Production of IL-6 under placebo, carbohydrate, and carbohydrate + glutamine at baseline, pre-exercise, post-exercise, and 2h post-exercise. Results expressed as mean ± SEM. Tukey's repeated measures analysis of variance, $P < 0.05$ and N = 15.

Table 1
Concentration of glutamine, glucose, cortisol, and erythropoietin (N = 15)

		Placebo	Carbohydrate	Carbohydrate + glutamine
Glutamine (mM)	Basal	0.65 ± 0.17	0.51 ± 0.08	0.49 ± 0.07
	Pre-exercise	0.51 ± 0.09	0.49 ± 0.07	0.44 ± 0.06
	Post-exercise	0.54 ± 0.07	0.54 ± 0.07	0.52 ± 0.10*
	After 2 h	0.45 ± 0.07†,‡	0.46 ± 0.07‡	0.46 ± 0.07
Glucose (mg/dL)	Basal	113.80 ± 7.73	114.28 ± 8.97	117.25 ± 7.56
	Pre-exercise	108.88 ± 9.69	112.23 ± 7.78	105.84 ± 10.93
	Post-exercise	148.35 ± 19.74*,†	169.16 ± 23.99*,†	151.94 ± 12.68*,†
	After 2 h	124.73 ± 16.75‡	144.09 ± 20.09*	128.34 ± 9.23*,‡
Cortisol (pg/mL)	Basal	1769.67 ± 371.85	1902.95 ± 326.12	1753.07 ± 313.48
	Pre-exercise	1520.71 ± 311.97	1778.11 ± 372.42	1749.69 ± 376.45
	Post-exercise	2031.85 ± 336.96*,†	2175.33 ± 299.57*	2051.52 ± 314.62*
	After 2 h	1390.56 ± 313.60†,‡	1809.20 ± 334.60‡	1613.67 ± 340.18‡
Erythropoietin (pg/mL)	Basal	60.83 ± 23.00	98.97 ± 46.97	89.19 ± 38.39
	Pre-exercise	59.52 ± 18.83	96.70 ± 39.01	75.46 ± 34.66
	Post-exercise	84.81 ± 24.89†	85.49 ± 30.84	96.48 ± 34.21
	After 2 h	95.43 ± 31.74‡	167.70 ± 70.45*,†	130.52 ± 60.80

Concentration of glutamine, glucose, cortisol, and erythropoietin under placebo, carbohydrate, and carbohydrate + glutamine at baseline, pre-exercise, post-exercise, and 2 h post-exercise. Results expressed as mean ± SEM. $P < 0.05$.
*Different from pre-exercise.
†Different from baseline.
‡Different from post-exercise.

reduction in IL-1 and TNF production at the same time, as well as the increase in post-exercise Gln concentration.

The reduction in SaO_2% 2 h after exposure to hypoxia in the three groups of men confirmed the efficiency of the hypoxia model used in this study and corroborated with previous studies [18]. The reduction in SaO_2% resulted in an increase in EPO concentration, another classic hypoxia-induced response [19]. However, 2 h after exercise the CHO + Gln supplementation increased SaO_2% compared with placebo, indicating that supplementation may contribute to attenuating the reduction in SaO_2% and avoiding the increase in EPO.

It has been suggested that the increased availability of Gln observed post-exercise may interfere with the central synthesis of Gln, an excitatory neurotransmitter that stimulates ventilation, contributing to the recovery of SaO_2% [20]. Another factor to consider in the CHO + Gln condition is the joint action of the two supplements [21], contributing to increased ventilation through different pathways [20].

The relationship between innate immune response and exercise in hypoxia is little known. The present results results show that hypoxia and exercise were not able to alter cytokine production. The decreased of IL-1 production 2 h after exercise in placebo may indicate a possible anti-inflammatory effect of exercise. However, a decrease in IL-1β and TNF-α production was observed in the CHO + Gln condition 2 h after exercise compared with baseline. The effects of hypoxia on cytokine production by monocytes are still controversial. Two hours of exposure at 5500 m reduced TNF-α production [8]. On the other hand, monocytes in culture (1% oxygen) for 18 h increased TNF-α synthesis [22]. These contradictions can be explained by the different methodologies used.

Initially, we thought that neuroendocrine changes could explain this pattern. In fact, neuroendocrine pathways, specifically the sympathoadrenal pathway, is activated in response to acute and chronic hypoxia, especially catecholamines and cortisol secretion [23]. In fact, both hormones are associated with cellular immunosuppression and alteration in the production and release of cytokines [1,4,6]. However, we found an increase in cortisol concentration after exercise that was reversed after 2 h of recovery in the three trials studied. If on the one hand these results show that the increase in cortisol caused by exercise is in agreement with previous studies [24], CHO supplementation during exercise was not able to inhibit or attenuate the increase in cortisol, as demonstrated in normoxia [25], despite the increase in glycemia observed in the three trials studied.

The majority of the cellular modifications and adaptations to hypoxia may be mediated by HIF-1, along with several other cellular mechanisms, such as mitochondrial generation of reactive oxygen species (ROS) or activation of nuclear factor (NF)-κB [26]. Under conditions of hypoxia, HIF-1 α accumulates in the cytoplasm and is inhibited in activated monocytes [27]. Additionally, monocytes incubated in hypoxia (1% oxygen) and normoxia (20.9% oxygen) for 1, 6, 16, and 24 h presented no differences in relation to the protein expression of HIF-1α, HIF-2α, and HIF-3α [28].

Additionally, the present results demonstrated that exercise realized in placebo was not able to modify the ability of monocytes to respond to LPS stimulation. Among the justifications, we suggest that the increase in catecholamines post-exercise may modify the relationship between the different monocyte groups, altering the inflammatory profile, due to the possible predominance of non-classical monocytes, which differ from the other monocytes by different expression of Toll-like receptor-4 [29].

Classical studies show the importance of CHO supplementation alone in preventing the immunosuppressive effects of strenuous exercise [25,30]. Gln, per se, may play an important role in modulating the immune response as leukocytes use Gln for energy production and synthesis of macromolecules [11,31]. Additionally, it has been suggested that the association of two or more forms of supplementation may have stronger effects than only one strategy [9]. With this in mind, we offered Gln supplementation for 6 d and on the day of exercise we associated CHO supplementation during exercise and recovery.

Regarding the Gln supplementation, unlike other studies in which supplementation was offered after the recovery period, we chose to offer it on the 6 d before exercise to guarantee a greater endogenous Gln stock and, consequently, higher Gln concentration inside the cell and slightly reduced in the bloodstream compared with placebo.

Two hours after exercise, Gln concentration was decreased in placebo and CHO, confirming a previous study [32]. On the other hand, we observed an increase in Gln concentration immediately after exercise in CHO + Gln. It is known that the functions of monocytes may be influenced by nutritional aspects [33], notably during physical exercise [34]. Activation of monocytes exposed to LPS results in a significant increase in Gln uptake and utilization [35]. Additionally, increased secretion of TNF-α and IL-6 by LPS-stimulated macrophages is

dependent on the availability of extracellular Gln [36,37]. We suggest that the increased availability of Gln after exercise due to CHO + Gln supplementation may have contributed to reduced production of IL-1β and TNF-α due to the anti-inflammatory effects of Gln.

It is possible that Gln may have prevented the expression of proinflammatory cytokines by inhibiting the NF-κβ pathway [38]. This fact can be explained by the ability of Gln to increase the amount of intracellular reduced glutathione, reducing the activity of kinases sensitive to the redox state [39]. Thus NF-κβ remains inactive associated with Ikβ in the cytoplasm.

We concluded that supplementation with combined CHO and Gln reduced the production of IL-1 and TNF by monocytes 2 h after exercise in hypoxia similar to 4500 m and increased SaO2% 2 h after strenuous exercise. These results create an opportunity for new studies on the theme as they suggest the potential role of combined supplements to attenuate the effects of hypoxia and frequent inflammation in hypoxic environments.

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